

290-Pos Board B169**Modulation Of Sequence-dependent Pausing Of RNA Polymerase By The Accessory Factor NusA: A Single-molecule Study**Jing Zhou¹, Kook Sun Ha², Arthur La Porta³, Robert Landick², Steven M. Block¹.¹Stanford University, Stanford, CA, USA, ²University of Wisconsin-Madison, Madison, WI, USA, ³University of Maryland, College Park, MD, USA.

Sequence-dependent pausing during transcription by RNA polymerase (RNAP) plays an essential role in the regulation of gene expression and is modified by timely interactions with a number of regulatory factors. In prokaryotes, NusA is a universally conserved accessory protein that is essential for cell viability and functions to modulate sequence-dependent pausing. Although NusA is known to both decrease the average rate of elongation and enhance certain types of pauses (e.g., the RNA hairpin-associated *his* pause), the kinetic details and mechanism underlying these effects are not well understood. We employed a dumbbell optical-trapping assay with high spatiotemporal resolution to probe directly the motion of individual molecules of RNAP transcribing a DNA template engineered to contain repeats of a sequence motif carrying the *his* pause. From individual transcriptional elongation records, the rates of entering pause states, the pause state lifetimes, and the pause-free elongation speeds can all be extracted. We found that the addition of NusA to the transcriptional assay significantly decreased the pause-free elongation speed while concurrently increasing the efficiency of entry into both long- and short-lifetime pauses. Studying these effects as a function of the applied load led to a quantitative kinetic scheme for elongation and pausing. In this model, the binding of NusA to RNAP functions equivalently to exerting a hindering load of ~19 pN on RNAP with respect to the DNA. We therefore speculate that the mechanism of NusA may be to modulate RNAP elongation in a mechanochemical sense.

291-Pos Board B170**Human TBP Bending of DNA Measured at the Single Molecule Level**Amanda E. Carpenter^{1,2}, Aaron R. Hieb¹, Meredith D. Betterton¹, James A. Goodrich¹, Thomas T. Perkins^{1,3}.¹University of Colorado, Boulder, CO, USA, ²JILA, Boulder, CO, USA,³JILA and National Institute of Standards and Technology, Boulder, CO, USA.

TATA binding protein (TBP) binds to DNA and bends it as a critical step of eukaryotic transcription. Human TBP induces an ~100° bend in the DNA helix. To help elucidate the role of DNA architecture in transcription, we developed a single-molecule, optical-trapping assay to study the TBP-induced bending of DNA. We hypothesized that DNA bending would lead to an apparent shortening of the DNA. The predicted signal size is small (~5 nm at 1 pN) and expected to decrease with increasing force. Moreover, the dynamics of TBP are slow, with a measured off-rate of ~10⁻² s⁻¹ at physiological salt conditions. To detect these small, infrequent events, we developed an actively stabilized optical trapping instrument. We achieved high spatiotemporal resolution [0.56 nm ($\Delta f = 0.01$ -1 Hz) over 200 s] at low force (1 pN) by developing a vertical optical trapping assay using short DNA tethers (92 nm) along with small beads (330 nm dia.). Detection of distinct TBP and TATA-box dependent signals required DNA molecules engineered to contain only one consensus TATA-box with no TATA-box like sequences in the flanking DNA. Significant non-specific binding to the glass surfaces was minimized by covalently attaching polyethylene glycol (PEG) to the glass. With these experimental procedures, we directly observed individual bending and unbending events. The TBP-dependent DNA conformational changes were dynamic on the timescale of tens of seconds at a [TBP] ≈ 20 nM and a force of 1 pN. The change in DNA extension (~5 nm) agreed well with theoretical predictions at 1 pN, but, unlike the simplest theory, showed little change in magnitude as the force was decreased. We expect that this assay will be broadly useful for studying DNA-protein interactions at high spatiotemporal resolution.

292-Pos Board B171**Mechanics of Transcription Elongation Through The Nucleosome Using Single-Molecule and Biochemical Methods**Pooja Gupta¹, Miroslav Tomschik¹, Andrey Revyakin², Jordanka Zlatanova¹.¹University of Wyoming, Laramie, WY, USA, ²QB3 Institute, University of California, Berkeley, CA, USA.

Transcription initiation and elongation in the eukaryotic nucleus occur in the context of chromatin. DNA wrapped around the histone core in the nucleosome constitutes a major obstacle to transcription. For transcription to occur, nucleosomes must be removed from the downstream DNA template. Numerous biochemical data indicate that this nucleosome disruption is a temporary event, with nucleosomes quickly reforming in the already transcribed portion of the template. We use a combination of single-molecule approaches (Magnetic

Tweezers) and biochemical methods to investigate the mechanics of transcription elongation through nucleosomes. Specifically, we have performed real-time transcription experiments with chromatin templates (208-18 DNA based) and T7 RNA Polymerase. The disassembly of 18 nucleosomes is expected to increase tether length by ~700 nm (each nucleosome is replaced by naked DNA of ~146 bp). We observe changes in tether length, the size of which corresponds to disassembly of single nucleosomes. We can say so far that transcription through the nucleosomal array results in disassembly of the nucleosomes. Some additional lengthening occurs as a result of changes in the geometry of the fiber (mutual disposition of nucleosomes, DNA entry-exit angles, etc.)

293-Pos Board B172**Regulation of Transcriptional Activity of Estrogen Receptor Alpha by Novel Splice Variants**

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Estrogen receptor alpha (ER α) mediates estrogen diverse actions on tissues. ER α gene has eight constitutively expressing exons and is known to have multiple isoforms generated by alternative initiation of transcription and splicing events like exon skipping. The functions of the variants thus generated are relatively unexplored. We have discovered two novel splice exons inserted between exon 5 and 6 of rat ER α that can add 18 and 14 amino acids consecutively within the estrogen binding domain of the receptor. The percentage of variant transcript expression with respect to constitutive ER α is higher in heart compared to ovary, uterus, brain, and intestine. The proportion of isoform/constitutive ER α expression increased with animal growth from prenatal to adulthood but did not further increase with aging (28 mo old). Inclusion of these exons yields a receptor with a much less binding capacity for estrogen and decreased nuclear to cytosolic localization. These variants are also less efficient in binding to the estrogen responsive element (ERE) and failed to transcriptionally activate promoters (mSlo and KCNE2) containing EREs. Moreover, the variant receptors exhibit a dominant negative effect on the transcriptional stimulatory activity of the wild type receptor. Studies on the mechanism of the dominant negative effect revealed that variant receptors do not significantly inhibit ligand binding, nuclear localization, or ERE-binding of the wild type receptor. However, the negative effect on the KCNE2 promoter could be nullified by coexpression of steroid receptor coactivators (SRCs), indicating that the variants inhibit wild type receptor activity probably by displacing SRCs from the pre-initiation complex. Thus, a naturally synthesized isoform of ER α regulates the wild type receptor function and may also contribute to the diverse action of estrogen observed in tissues. Supported by AHA and NIH.

294-Pos Board B173**Modeling Transcription Initiation By Bacterial RNA Polymerase Marko Djordjevic.**

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Bacterial RNA polymerase (RNAP) is the central enzyme of gene expression, and initiation of transcription by RNAP is both the first step and a major point in regulation of gene expression. As a first step of transcription initiation, RNAP binds to double stranded DNA and opens the two strands of DNA, which is referred to as the open complex formation. A question of how the open complex is formed still remains open, despite significant experimental efforts over the last two decades [1].

We develop the first quantitative model of the open complex formation by bacterial RNAP, which provides a theoretical framework needed to analyze the assembled experimental data. The model is based on statistical physics and establishes an explicit relationship between the rate of transcription initiation and biophysical properties of promoter sequence and promoter-RNAP interactions [2]. We compare our model with both biochemical measurements and genomics data and report a very good agreement with the experiments, with no free parameters used in model testing. Qualitatively, our model strongly supports a hypothesis by which the open complex formation is a two step process, where the transcription bubble is first formed in the -10 region and consequently extended to the transcription start site. Quantitatively, our results allow efficient estimation of promoter kinetic parameters for any given DNA sequence, as well as 'engineering' of promoter sequences with the desired kinetic properties. Finally, we discuss how our biophysical model can be used to investigate kinetic properties of promoter sequences on a genome wide scale [3].

[1] Young, B.A., T.M. Gruber, and C.A. Gross. *Science* 303:1382-1384 (2004).[2] M. Djordjevic and R. Bundschuh. *Biophysical Journal* 94: 4233-4248 (2008).

[3] M. Djordjevic. Quantitative analysis of promoter kinetic properties on a genome-wide scale (to be submitted, 2008).